

with the appropriate sized insert in the correct orientation relative to the T7 promoter was identified by separate digestion with NheI, NcoI or XhoI. By subcloning fragment in the NheI site of pET9d DN, the putative precursor polypeptide was joined to the ATG codon downstream from the T7 promoter and bacterial Shine & Dalgarno sequence provided by pET9d DN. This NheI site changed the nucleotide sequence of the putative N-terminus of the precursor protein from ~~ATG GCG CTC CGC~~ to ~~ATG GCT AGC CTC CGC~~, which changed the encoded ~~Met-Ala-Leu-Arg~~ amino acid sequence to ~~Met-Ala-Ser-Leu-Arg~~. The insert of this clone was sequenced entirely and the clone was assigned number pDAB432D

The precursor maize  $\Delta 9$  desaturase was expressed in *E. coli* BL21(DE3) (Novagen Inc., Madison, WI). For small scale expression, 1  $\mu$ g of plasmid pDAB432D was transformed into 200  $\mu$ L CaCl<sub>2</sub> competent cells and plated on two LB plates containing kanamycin at 25  $\mu$ g/mL. Following overnight incubation at 37° C colonies were scraped off the plate and resuspended in 10 mL of LB Broth containing kanamycin (50  $\mu$ g/mL) and isopropyl- $\beta$ -D-thiogalactoside (IPTG) at 1.0 mM. Cells were allowed to express proteins for 3 hr during vigorous shaking at 37° C. Cells were harvested by centrifugation at 3000 rpm for 10 min at 4° C. The cell pellet was frozen and thawed twice in dry ice-ethanol to improve cell lysis. Next, the cell pellet was resuspended in 1.0 mL of lysis buffer (10 mM Tris HCl pH 8.0, 1.0 mM EDTA, 150 mM NaCl, 0.1% Triton X 100, 100  $\mu$ g/mL DNaseI, 100  $\mu$ g/mL RNaseH, 1.0 mg/mL lysozyme) and incubated at 37° C until it was no longer viscous. Soluble proteins were separated from aggregated denatured proteins by centrifugation at 4° C for 10 min. The insoluble pellet was resuspended in about 300  $\mu$ L of the above lysis buffer. Both fractions had an approximate final volume of 0.5 mL.

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